amount of intestinal alkaline phosphatase equivalent to 50 units of activity[1] and allow to stand until the phosphatase has dissolved completely. Place the centrifuge tube into a water bath at 37° C ± 2° C for 2.5 hours. After the 2.5-hours hydrolysis, allow the solution to cool.

(ii) *Calculations.* Calculate the clindamycin content as follows:

$$\text{Clindamycin content per milliliter} = (R_u \times W \times_s \times d \times f)/(R_s \times V)$$

where:

R_u=Area of clindamycin sample peak/Area of internal standard;
R_s=Area of clindamycin standard peak/Area of internal standard;
W_s=Weight of clindamycin working standard in milligrams;
d=Dilution factor;
f=Potency of clindamycin working standard in milligrams of clindamycin per milligram;
V=Volume of sample in milliliters.

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted drug.

[46 FR 2997, Jan. 13, 1981. Redesignated at 54 FR 38224, Sept. 15, 1989]

§ 453.522b Clindamycin phosphate gel.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clindamycin phosphate gel contains clindamycin phosphate in a suitable and harmless vehicle. Each gram contains clindamycin phosphate equivalent to 10 milligrams of clindamycin activity. Its clindamycin content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of clindamycin that it is represented to contain. Its pH is not less than 4.5 and not more than 6.5. It passes the identity test. The clindamycin phosphate used conforms to the standards prescribed by § 453.22(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification: samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The clindamycin phosphate used in making the batch for clindamycin content, microbiological activity, moisture, pH, crystallinity, and identity.

(B) The batch for clindamycin content, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The clindamycin phosphate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(B) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Clindamycin content (High performance liquid chromatographic assay).* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 210 nanometers, a 25-centimeter long x 4.6-millimeter ID column packed with microparticulate (5 to 10 micrometers in diameter) reversed phase octysilane hydrocarbon bonded silica packing material, a flow rate of about 1.0 milliliter per miunute, and a known injection volume of between 10 and 20 microliters. The retention time of clindamycin phosphate, and clindamycin are approximately 6 and 9 minutes, respectively. Reagents, working standards and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *0.1M Potasium phosphate monobasic buffer.* Dissolve 13.61 grams of potassium phosphate monobasic in 775 milliliters of water. Adjust the pH to 2.5 with phosphoric acid. Further dilute with water to a volume of 1,000 milliliters.

(B) *Mobile phase.* Mix 225 milliliters of acetonitrile and 775 milliliters of 0.1*M* potassium phosphate, pH 2.5 buffer (225:775). Filter through a suitable filter capable of removing particulate matter greater than 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(A)

[1] Defined such that 50 units hydrolyzes at least 20 micromoles of a clindamycin phosphate authentic sample under the assay conditions described in § 436.304 of this chapter.

Working standard solution. Dissolve an accurately weighed portion of the clindamycin phosphate working standard with sufficient mobile phase (prepared as directed in paragraph (b)(1)(i)(B) of this section) to obtain a solution containing 200 micrograms of clindamycin activity per milliliter.

(B) *Sample solution.* Accurately weigh and transfer approximately 2.0 grams of the sample into a 100-milliliter volumetric flask. Dilute to volume with sufficient mobile phase (prepared as directed in paragraph (b)(1)(i)(B) of this section) and shake vigorously for 30 minutes. Centrifuge a portion of the solution and if necessary filter a few milliliters of the centrifuged solution through a 2-micron millipore filter, type BS.

(C) *Resolution test solution.* Place 15 milligrams each of clindamycin phosphate and clindamycin hydrochloride in a 25-milliliter volumetric flask and dissolve and dilute to volume with mobile phase and mix well. Use this solution to determine the resolution factor.

(iii) *System suitability requirements*—(A) *Asymmetry factor.* Calculate the asymmetry factor (A_s), measured at a point 5 percent of the peak height from the baseline as follows:

$$A_s = \frac{a+b}{2a}$$

where:

a = Horizontal distance from point of ascent to point of maximum peak height; and

b = Horizontal distance from the point of maximum peak height to point of descent.

The asymmetry factor (A_s) is satisfactory if it is not more than 1.3.

(B) *Efficiency of the column.* From the number of theoretical plates (n) calculated as described in § 436.216(c)(2) of this chapter calculate the reduced plate height (h_r) as follows:

$$h_r = \frac{(L)(10{,}000)}{(n)(d_p)}$$

where:

L = Length of the column in centimeters;

n = Number of theoretical plates; and

d_p = Average diameter of the particles in the analytical column packing in micrometers.

The absolute efficiency (h_r) is satisfactory if it is not more than 15.

(C) *Resolution factor.* The resolution factor (R) between the peak for clindamycin phosphate and the peak for clindamycin (hydrochloride) in the chromatogram of the resolution test solution is satisfactory if it is not less than 6.0.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_R in percent) of 5 replicate injections of the working standard solution (prepared as directed in paragraph (b)(1)(ii)(A) of this section is satisfactory if it is not more than 2.5 percent. If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(iv) *Calculations.* Calculate the clindamycin content as follows:

$$\text{Milligrams of clindamycin per gram} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u = Area of the clindamycin phosphate peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s = Area of the clindamycin phosphate peak in the chromatogram of the clindamycin phosphate working standard;

P_s = Clindamycin activity in the clindamycin phosphate working standard solution in micrograms per milliliter; and

d = Dilution factor of the sample.

(A) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted gel.

(B) *Identity.* The high-performance liquid chromatogram of the sample determined in paragraph (b)(1) of this section compares qualitatively to that of the clindamycin phosphate working standard.

[54 FR 38224, Sept. 15, 1989]

§ 453.522c Clindamycin phosphate lotion.

(a) *Requirements for certification*—(1) *Standards for identity, strength, quality, and purity.* Clindamycin phosphate lotion contains clindamycin phosphate in a suitable and harmless lotion vehicle, with one or more suitable and harmless emollients, buffers, and dispersants. Each milliliter contains clindamycin phosphate equivalent to 10 milligrams